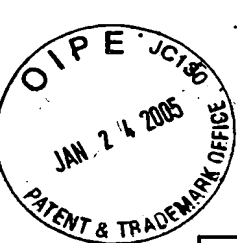


FIG. 1



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NO.	IDEAL CONDITION: ALL BASE AND F ADDUCTS FULLY CHARGED				IN PURE WATER	
	Charge on indicated moiety		Net charge		pH 7.0	
	NUCLEOBASE	F	NP PROBE	PPI-F	Change	Change
1	-3	-3	-9	-6	3	3.25
2	-3	-2	-8	-5	3	3.25
3	-3	-1	-7	-4	3	3.25
4	-3	0	-6	-3	3	3.25
5	-3	1	-5	-2	3	3.25
6	-3	2	-4	-1	3	3.25
7	-3	3	-3	0	3	3.25
8	-2	-3	-8	-6	2	2.26
9	-2	-2	-7	-5	2	2.26
10	-2	-1	-6	-4	2	2.26
11	-2	0	-5	-3	2	2.26
12	-2	1	-4	-2	2	2.26
13	-2	2	-3	-1	2	2.26
14	-2	3	-2	0	2	2.26
15	-1	-3	-7	-6	1	1.26
16	-1	-2	-6	-5	1	1.26
17	-1	-1	-5	-4	1	1.26
18	-1	0	-4	-3	1	1.26
19	-1	1	-3	-2	1	1.26
20	-1	2	-2	-1	1	1.26
21	-1	3	-1	0	1	1.26
22	0	-3	-6	-6	0	0.26
23	0	-2	-5	-5	0	0.26
24	0	-1	-4	-4	0	0.26
25	0	0	-3	-3	0	0.26
26	0	1	-2	-2	0	0.26
27	0	2	-1	-1	0	0.26
28	0	3	0	0	0	0.26
29	1	-3	-5	-6	-1	-0.74
30	1	-2	-4	-5	-1	-0.74
31	1	-1	-3	-4	-1	-0.74
32	1	0	-2	-3	-1	-0.74
33	1	1	-1	-2	-1	-0.74
34	1	2	0	-1	-1	-0.74
35	1	3	1	0	-1	-0.74
36	2	-3	-4	-6	-2	-1.74
37	2	-2	-3	-5	-2	-1.74
38	2	-1	-2	-4	-2	-1.74
39	2	0	-1	-3	-2	-1.74
40	2	1	0	-2	-2	-1.74
41	2	2	1	-1	-2	-1.74
42	2	3	2	0	-2	-1.74
43	3	-3	-3	-6	-3	-2.74
44	3	-2	-2	-5	-3	-2.74
45	3	-1	-1	-4	-3	-2.74
46	3	0	0	-3	-3	-2.74
47	3	1	1	-2	-3	-2.74
48	3	2	2	-1	-3	-2.74
49	3	3	3	0	-3	-2.74

FIG. 2

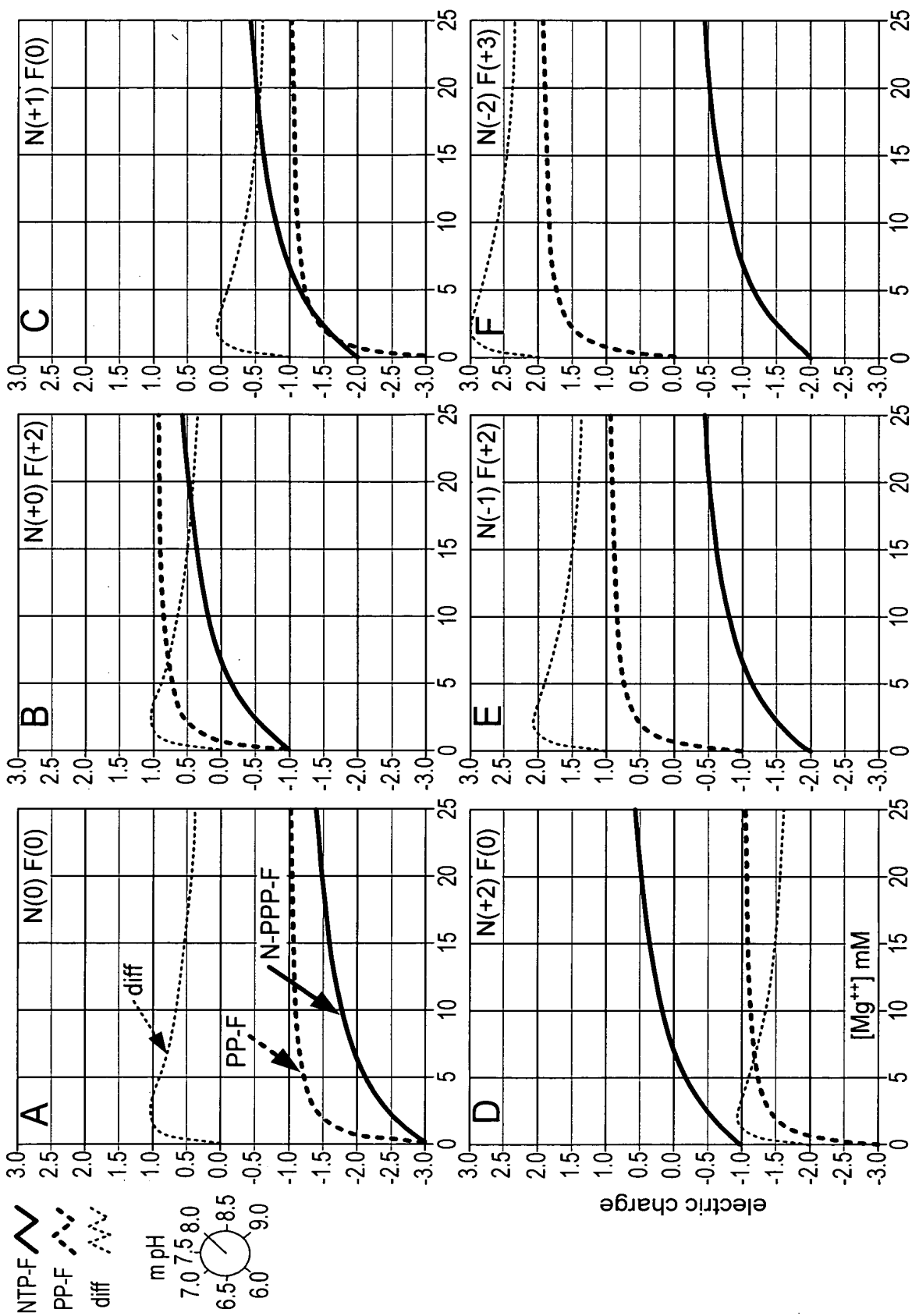


FIG. 3

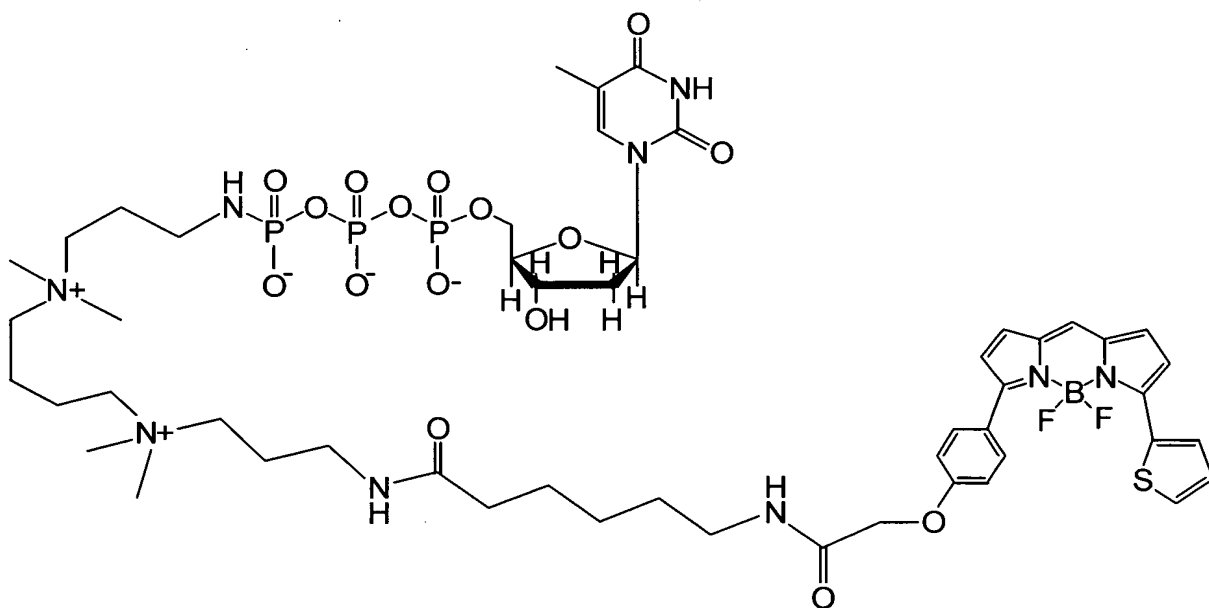


FIG. 4

Equilibrium Calculations For N-PPP-F

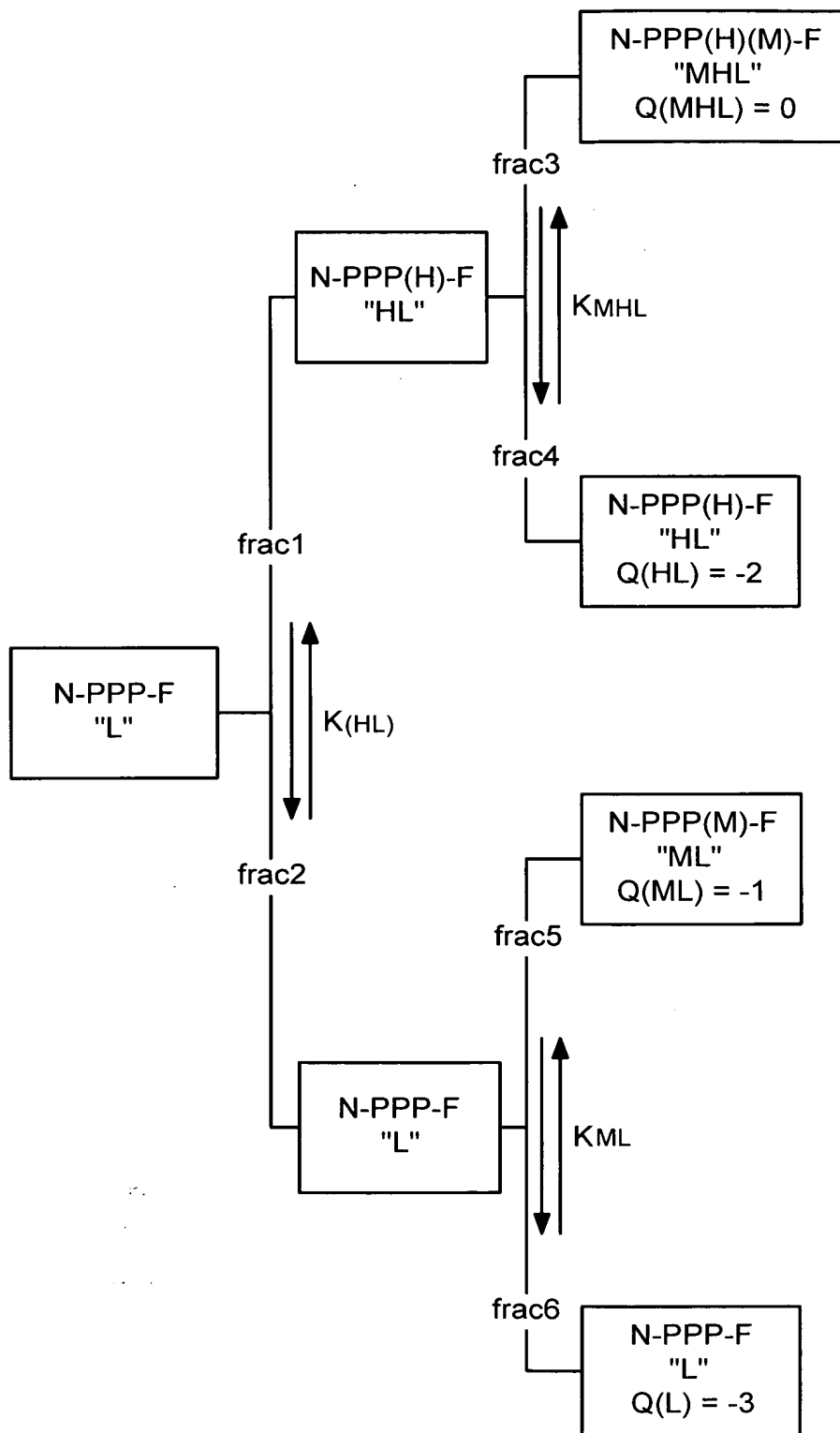


FIG. 5

Compound	Charge	Name	Structure
50	N = -2 F = +2	DBA-U-BQS-TAMRA X	
51	N = -2 F = +1	DBA-U-BQS-Oregon 500	
52	N = -1 F = +2	SUC-U-BQS-TAMRA X	

FIG. 6A

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53	N = +2 F = +1	PAA-A-BQS- Oregon 500	
54	N = +2 F = 0	PAA-U-BQS- Alexa 488	

FIG. 6B

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55	N = -1 F = +1	Suc-U- peptide+2- Oregon 500	
56	N = 0 F = 2	T-BQS- TAMRA X	
57	N = 0 F = 2	T-BQS- TAMRA	

FIG. 6C

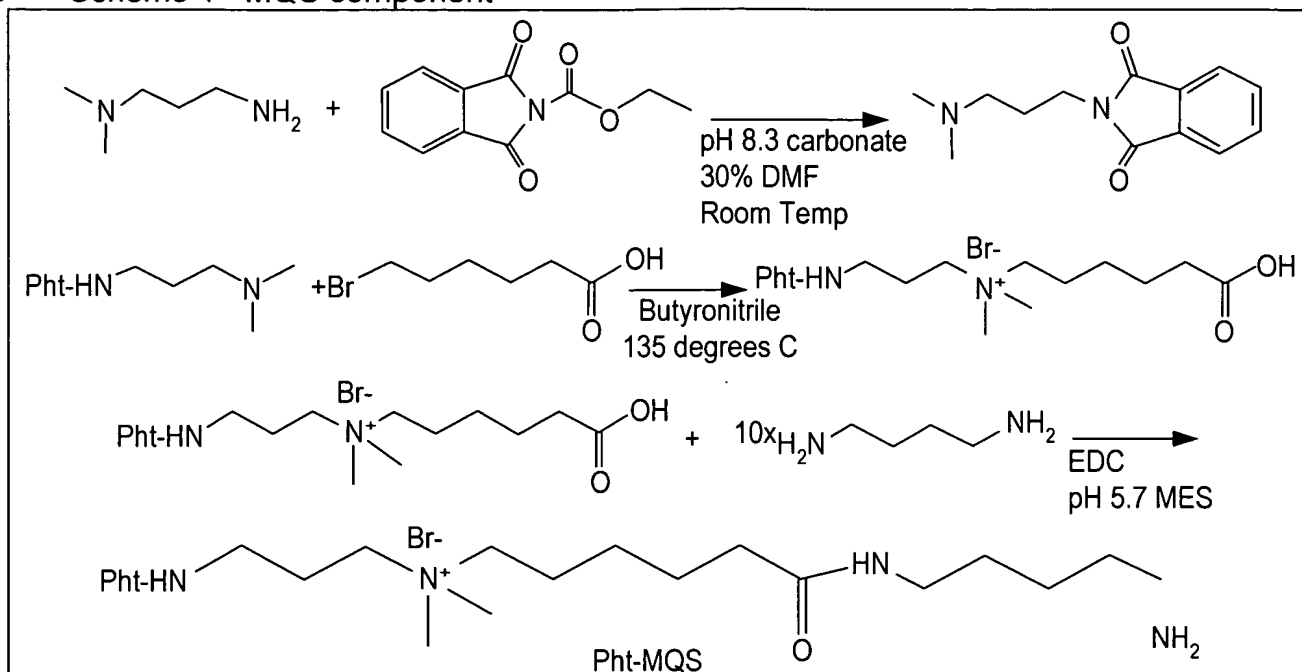
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58	N = +1 F = +1	AA-U-BQS- Oregon 500	
59	N = 0 F = +1	G-BQS- Oregon 500	
60	N = +2 F = +1	PAA-C-BQS- Oregon 500	

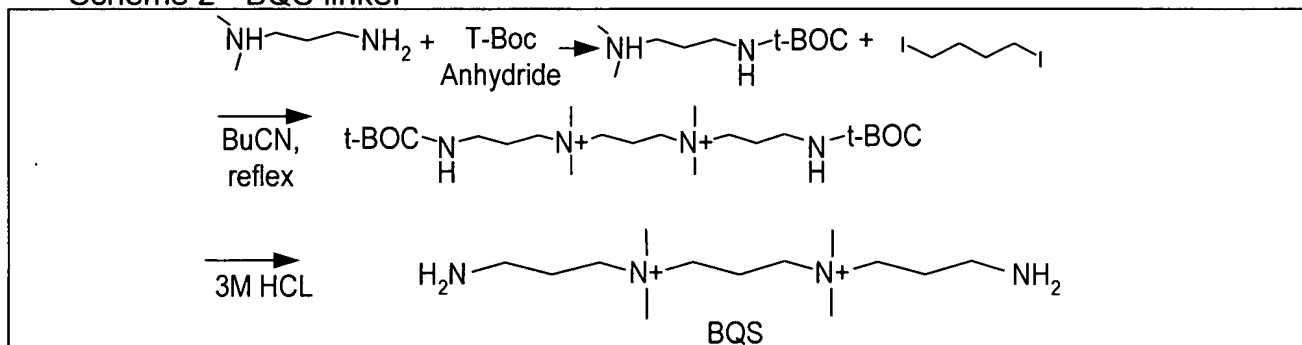
FIG. 6D

Scheme 1 - MQS component

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Scheme 2 - BQS linker



Scheme 3 - TQS linker

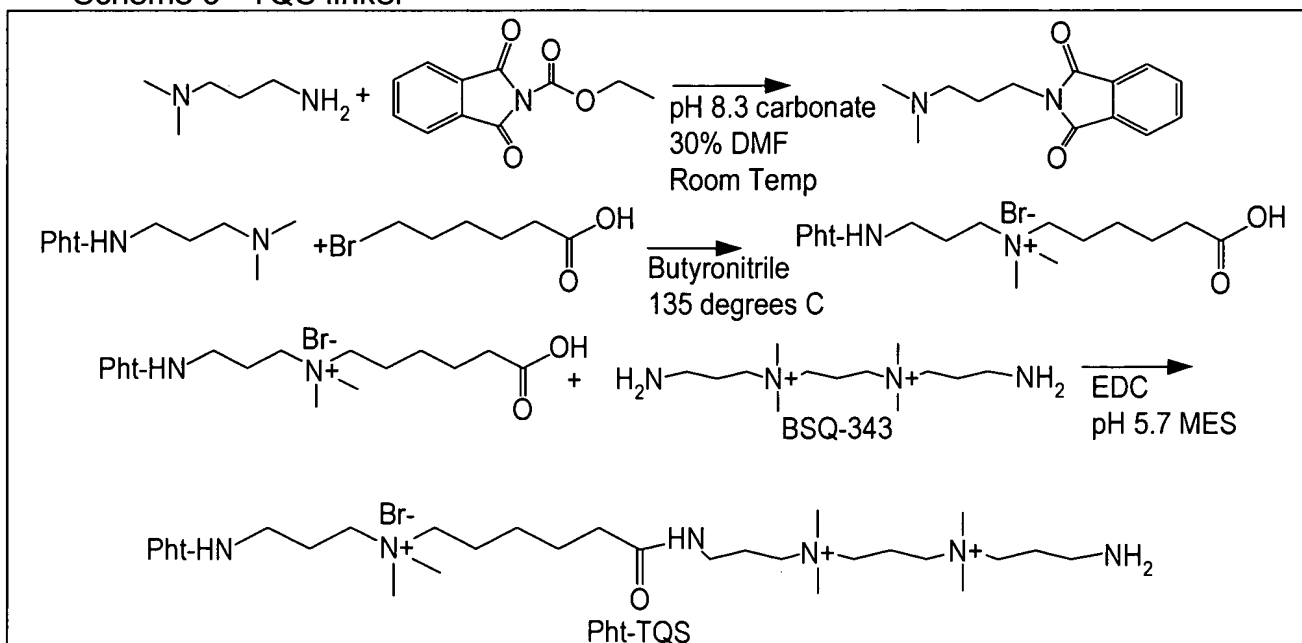
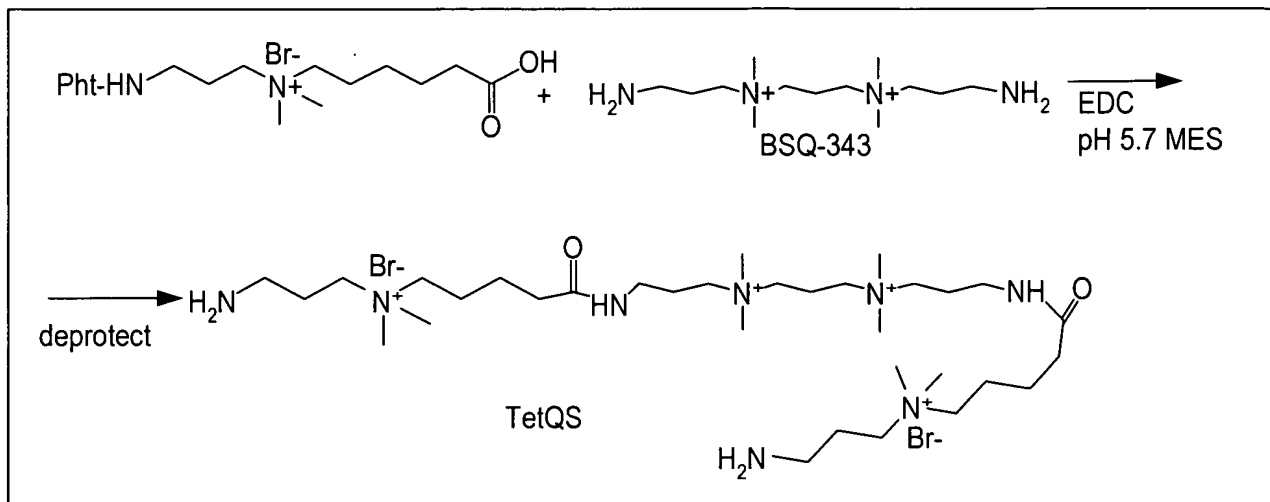


FIG. 6E

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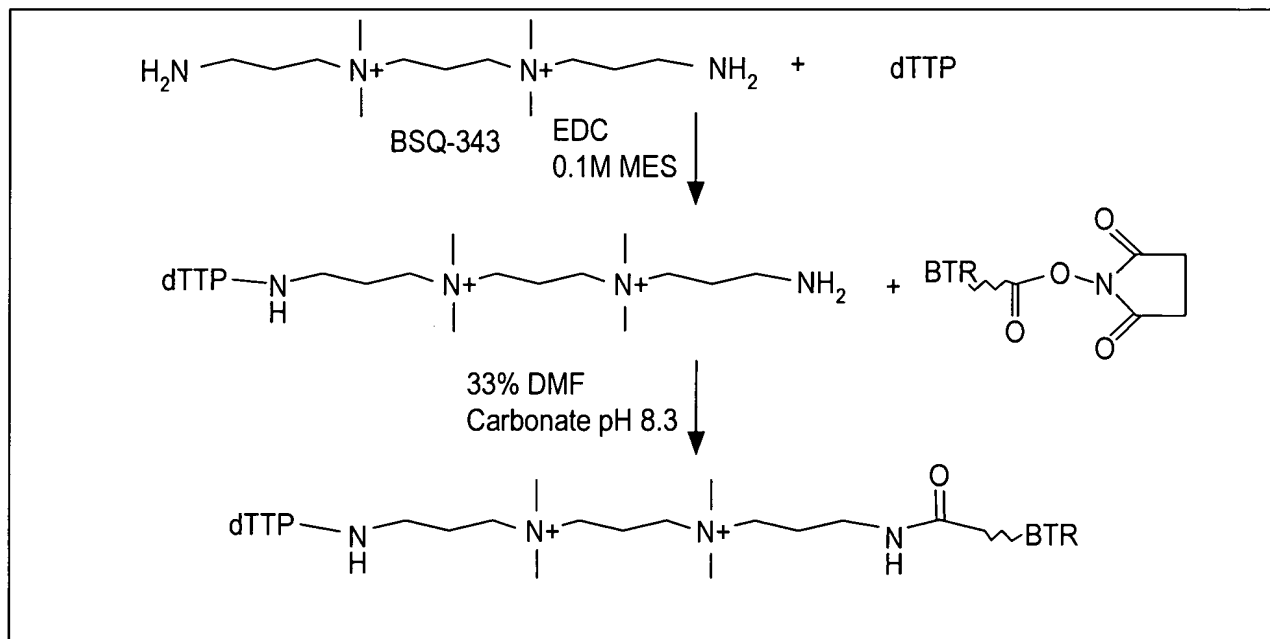
Scheme 4 TetQS linker



Scheme 5 - Protect AA-dUTP

Use same chemistry as in Scheme 1, except the amine is the aminoallyl group of AA-dUTP. We have shown that deprotection can be accomplished in 1M NaOH, room temp, 2 hours, without degrading triphosphates.

Scheme 6

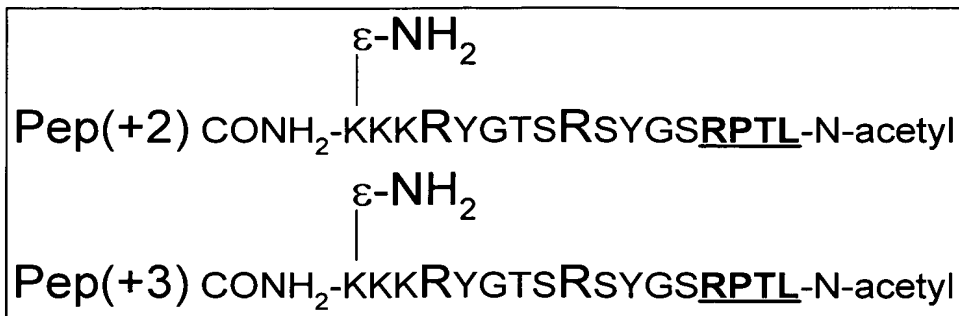


Coupling nucleotide, linker and dye (BTR is BodipyTR dye shown as the succinimide ester). We use this chemistry routinely to make γ -dNTPs (e.g, see cpd of Fig 13A)

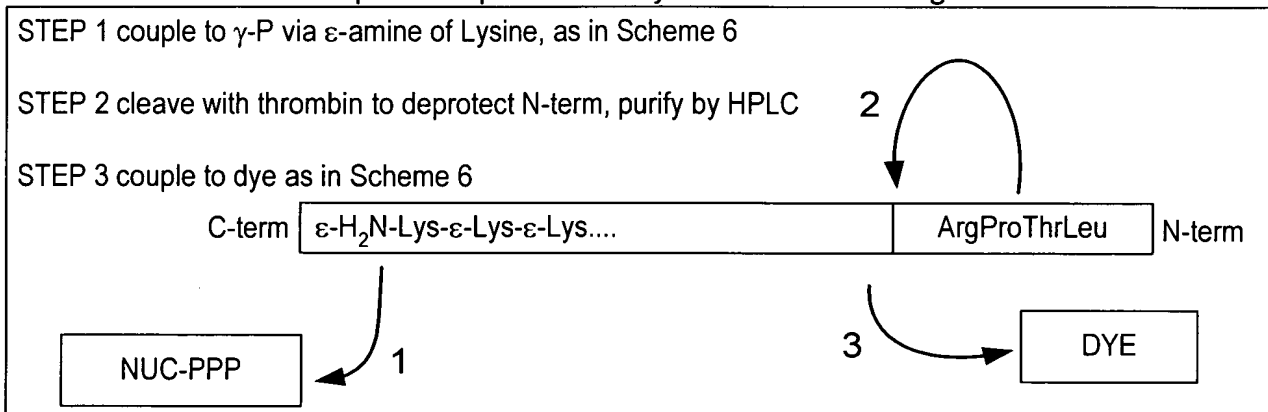
FIG. 6E (continued)

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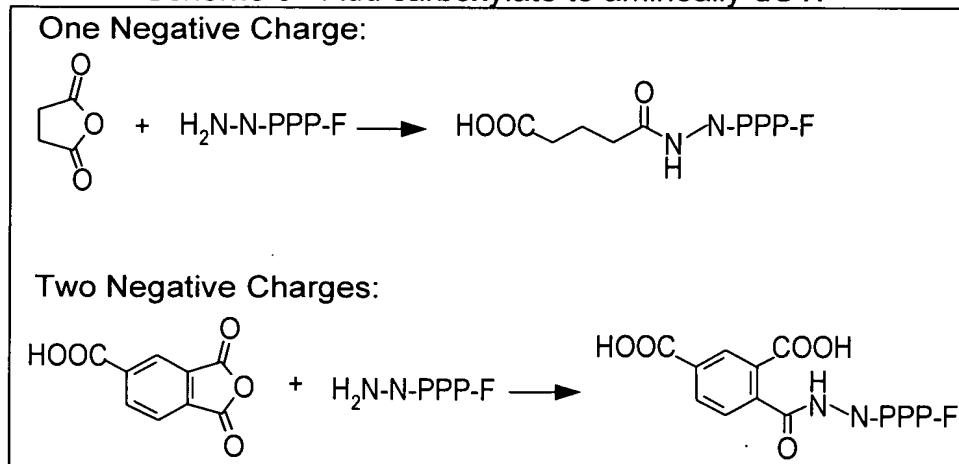
Scheme 7 - Peptide linkers (shown in C-to-N direction)



Scheme 8 - Peptide Deprotection By Thrombin Cleavage



Scheme 9 - Add carboxylate to aminoallyl-dUTP



Scheme 10 - g-dNTP With Carboxylated Base

1. $\text{NH}_2\text{-dU-PPP} + \text{Pht (of Scheme 1)} \longrightarrow$
2. $\text{Pht-NH-dU-PPP} + \epsilon\text{NH}_2\text{-KKK-pep-RPTL} \longrightarrow$
3. $\text{Pht-NH-dU-PPP-KKK-pep-RPTL} + 1\text{M NaOH} \longrightarrow$
4. $\text{NH}_2\text{-dU-PPP-KKK-pep-RPTL} + \text{anhydride (of Sche} \longrightarrow$
5. $(\text{COO}^-)\text{-dU-PPP-KKK-pep-RPTL} + \text{thrombin} \longrightarrow$
6. $(\text{COO}^-)\text{-dU-PPP-KKK-pep-NH}_2 + \text{SE-Dye} \longrightarrow$
7. $(\text{COO}^-)\text{-dU-PPP-KKK-pep-NH-Dye}$

FIG. 6F

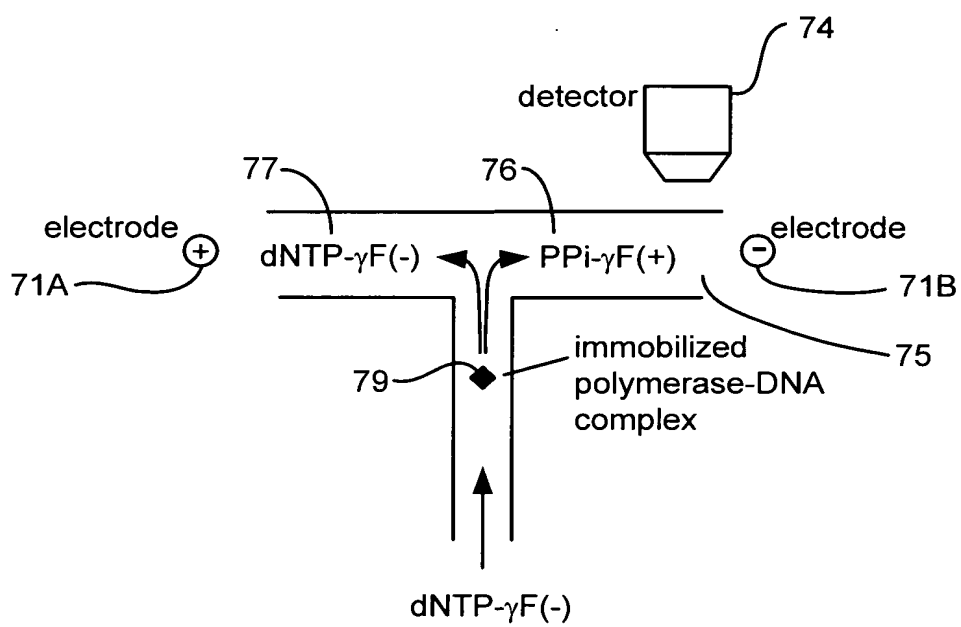


FIG. 7

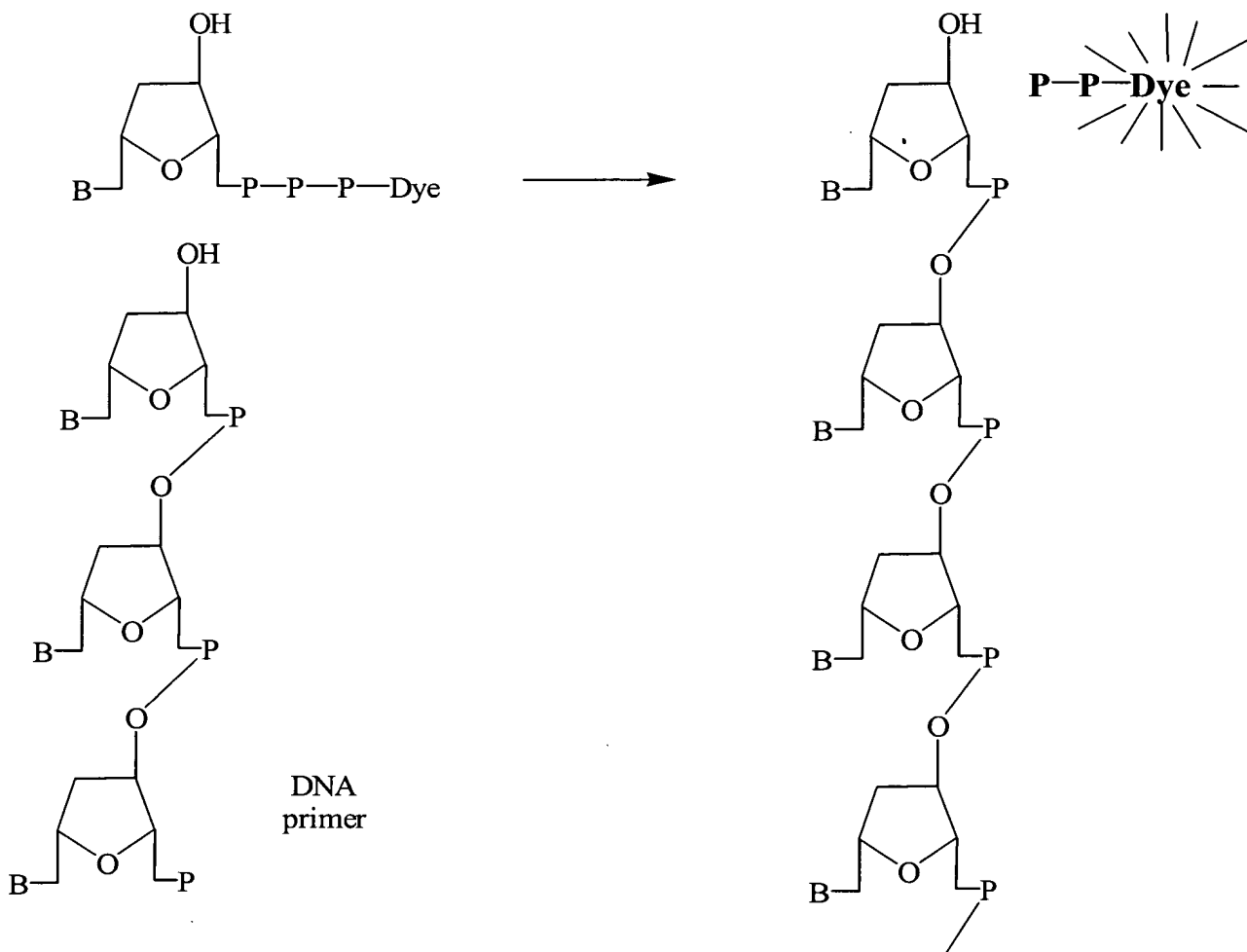


FIG. 8

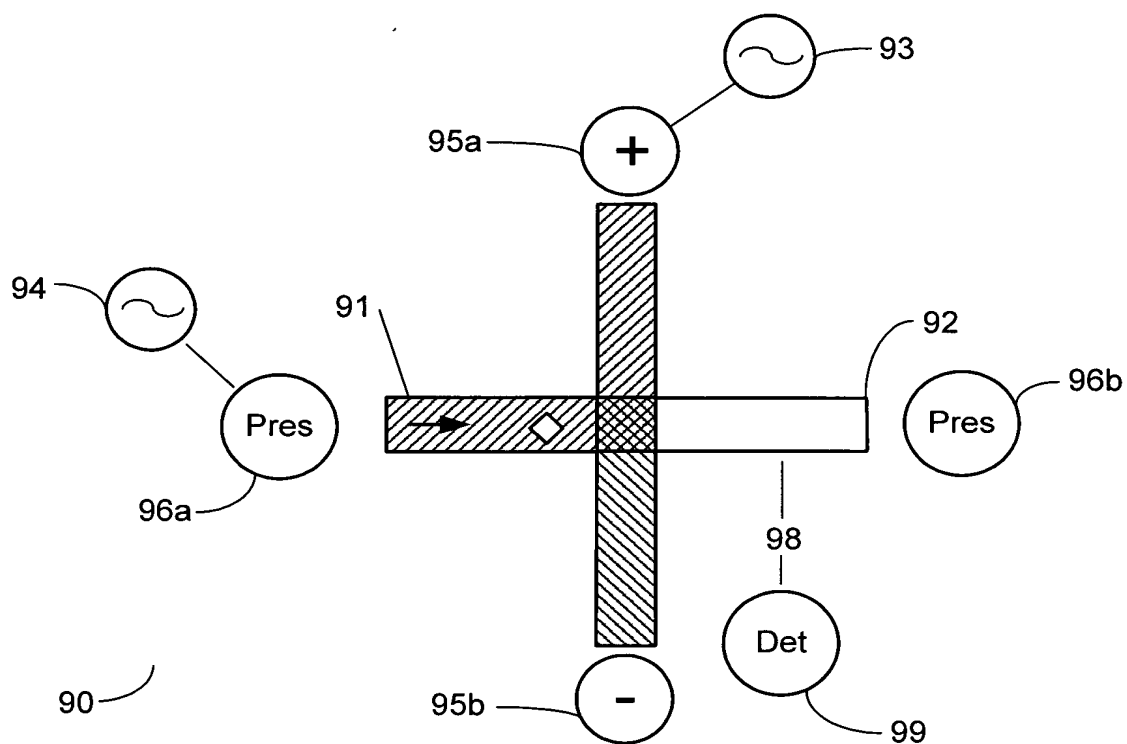


FIG. 9



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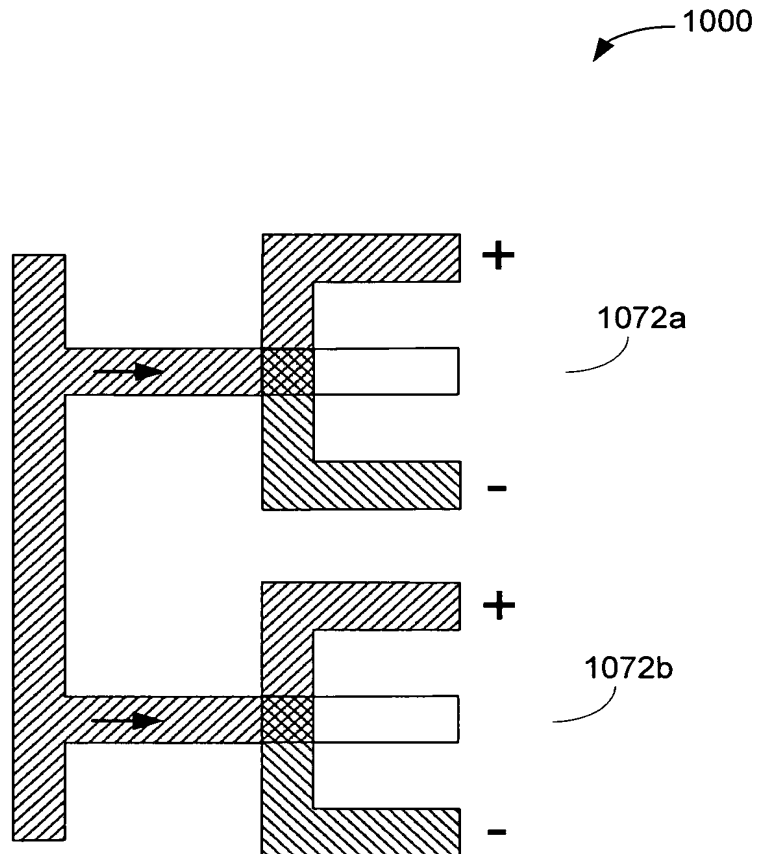


FIG. 10

Mg⁺⁺ Controls Electrophoretic Mobility of Nucleotides

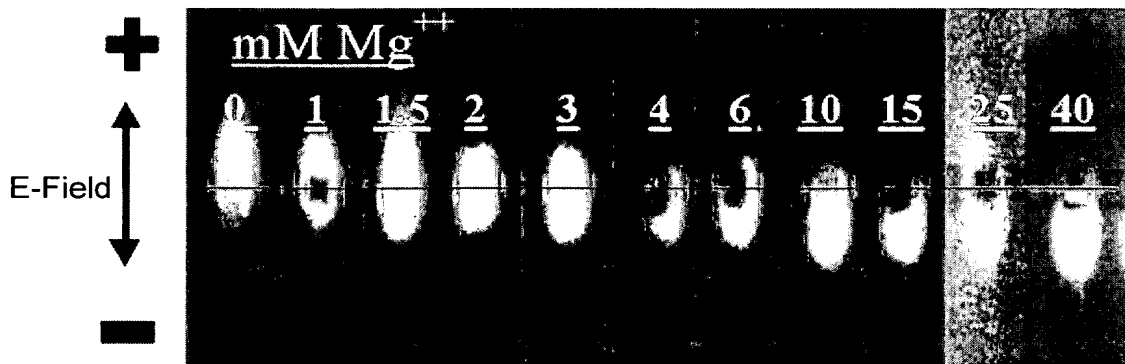
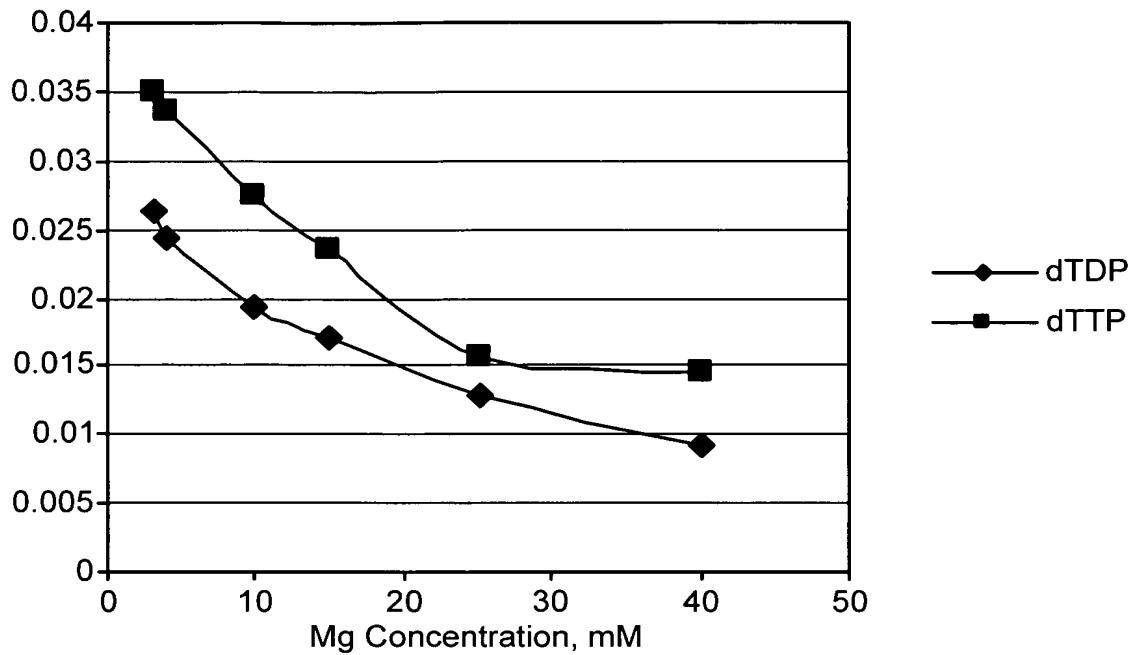


FIG. 11

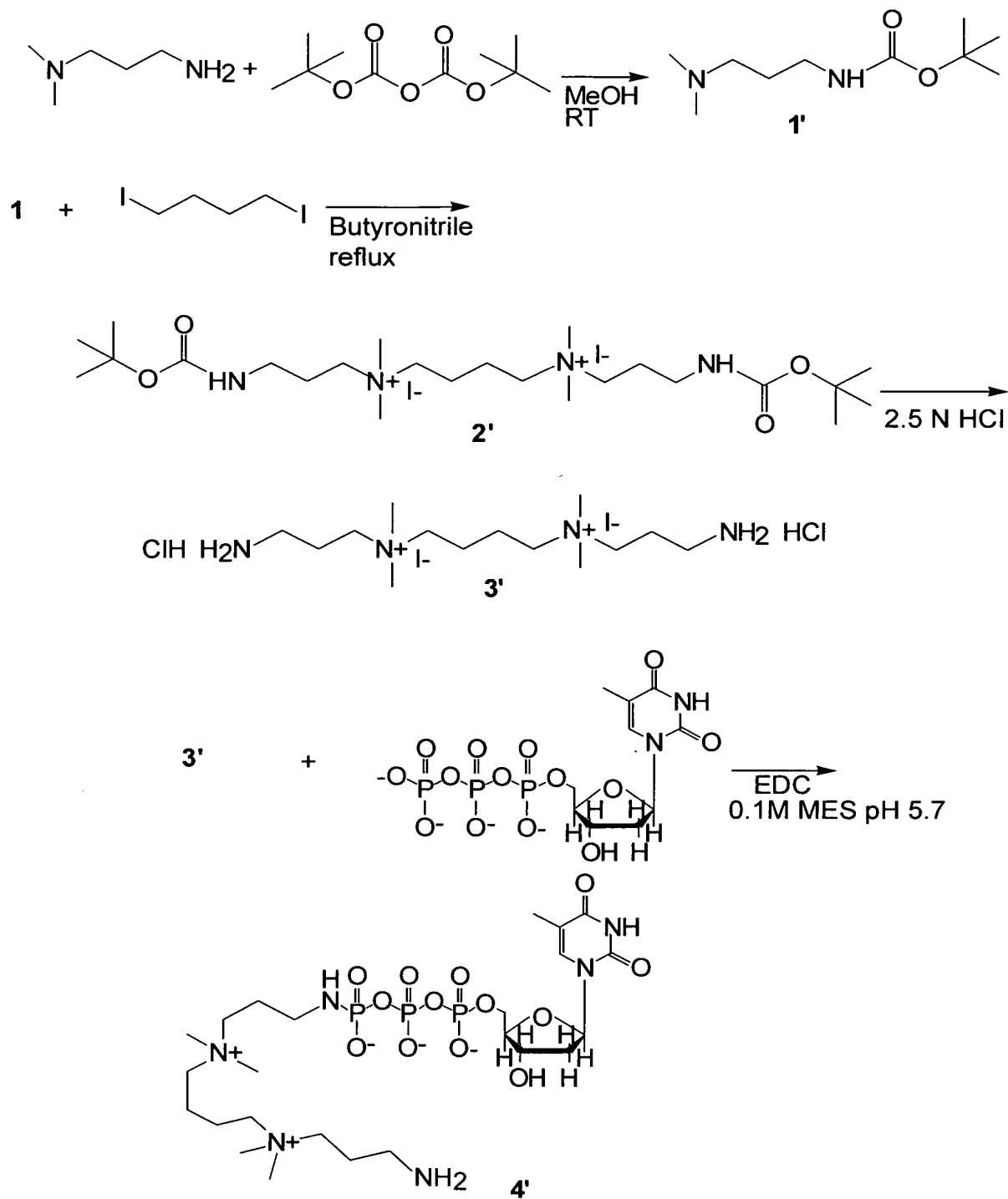


FIG. 12

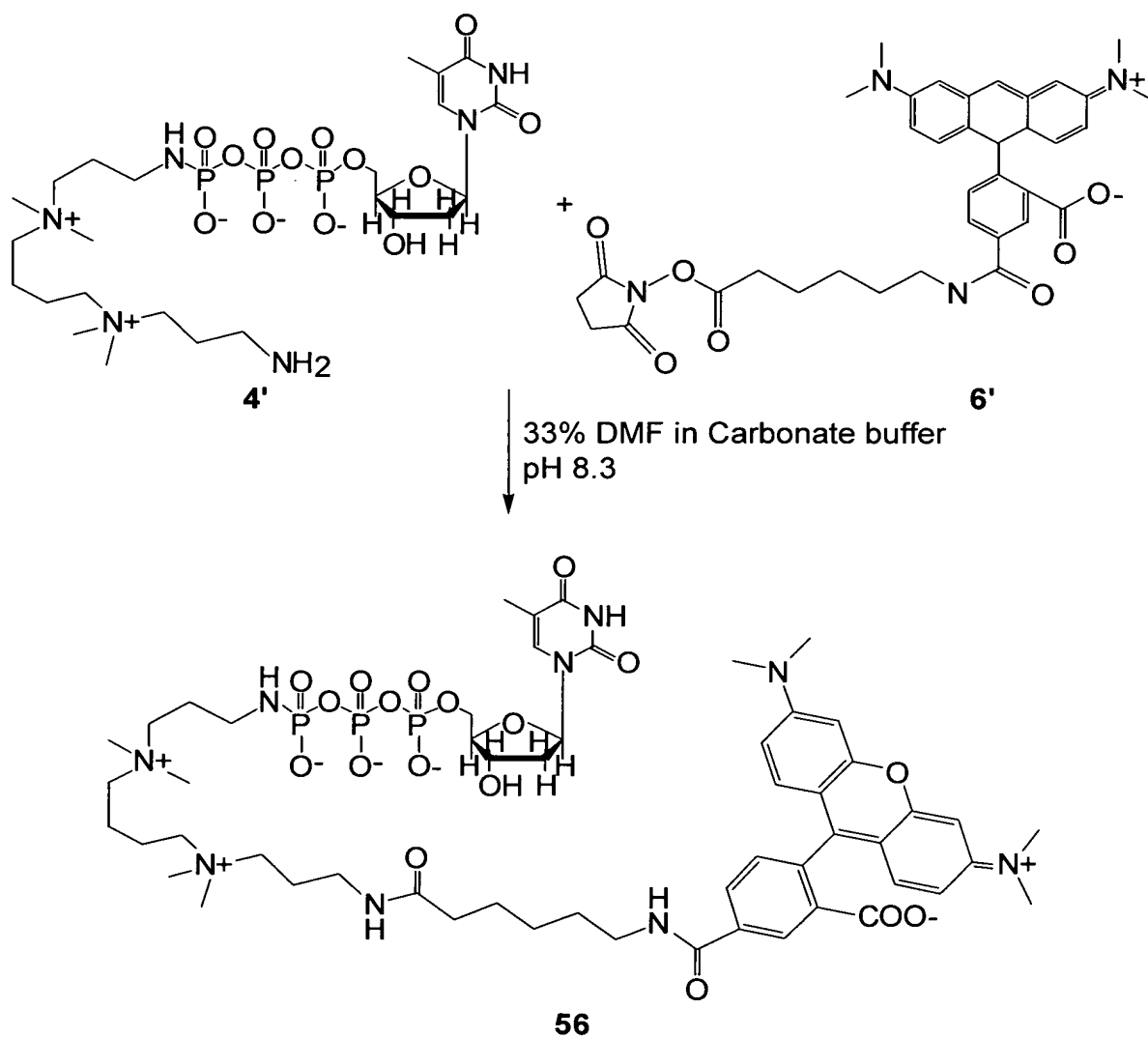


FIG. 12 (continued)